CONTAMINATION AND CONSUMPTION OF STORED WHEAT BY A CLOSED POPULATION OF *MUS MUSCULUS*

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ABSTRACT

A simulation of bulk storage of winter wheat infested with a closed population of house mice (*Mus musculus*) was used to develop and combine methods for assessing contamination and consumption losses in actual storage units. Several of the findings reported here seem obvious, however, verification by quantification was needed. The simulated storage was carried out for 150 days. The number of animals remained fairly constant during the study. A total of 109 kg of wheat with a surface area of 1.97 m^2 was used. Urine, feces and hair contamination were estimated for surface (upper 2.5 cm) wheat by location, and for total wheat stored. Wheat consumption was also determined. A simple method using plastic bags to examine samples permitted reliable estimates of hairs and droppings. A previously developed method for the analysis of urea is described and was used for determining the presence of urine on the wheat seeds. The mice consumed an average of 35 grams per day. During the 150 days, 65 percent of the surface seeds and 55 percent of all seeds were contaminated with urine. There were no significant effects on the number of urine-contaminated seeds with respect to location within the storage area. Duncan's multiple range tests showed no location patterns evident for droppings or hair. Nevertheless, identical locations were found for the six most active and six least active sites for both droppings and hairs. Corners were contaminated the most, followed by edges, while the center of the stored wheat was the least contaminated.

INTRODUCTION

Rodents contaminate and consume vast quantities of stored grains throughout the world. However, there are few data concerning the quantity of grains contaminated and consumed. This lack of quantitative information is not only true for countries and municipalities, but also for individual warehouses or storage units. Available data are broad undocumented estimates and invalid extrapolations. Jackson (1977) tabulated these figures and discussed their inadequacies but concluded: "In reality, the specific statistics is of relatively little consequence". Although this conclusion may have validity on a worldwide scale, we believe that precise loss statistics for storage units is essential to the determination of cost-benefit ratios for control programs and also as measures of efficacy in the development and application of damage control methods. The work described here was undertaken as a first step in developing a loss assessment technique usable at individual storage units.

MATERIALS AND METHODS

A mouse (*Mus musculus*) population, which consisted initially of eight nonpregnant females and four males (individually identifiable by toe clip), was established in a small brick and cement storage room at the Denver Wildlife Research Center. All potential mouse entrances and exits were eliminated. The room has a floor space of 10.5 m^2. Sufficient harborage, bedding, and nesting materials were placed evenly around the perimeter of the room to meet the needs of a growing mouse population. After introduction of the 12 mice, baseline data on water and food (hard winter wheat) consumption were recorded each Tuesday and Friday for a four-week period. The collected data indicated stability of the 12 mouse population during the acclimation period, and also indicated the mean water and food consumption per mouse.

After the initial four-week period, all wheat was removed and a frame with inside dimensions of 236 cm length x 8.6 cm diameter x 83 cm width was centered in the room. The frame was filled with 109 kg of hard winter wheat to a depth of 6.4 cm with a surface area of 1.97 m^2 to simulate bulk storage. Water remained *ad libitum* and consumption was recorded twice weekly, as before, to monitor population changes. This study was conducted from late May through late October. The room and contents remained undisturbed for 150 days except for the replenishment of water.

After this period, all mice were removed, and the population, the sex ratio, and the survival rate (of the original 12 mice) were determined. Samples of wheat from the upper 2.5 cm were taken from the frame at points (rows and columns) shown in Tables I and II. Two samples of approximately 25 g by volume were taken from each row and column position shown in the tables in order to determine if location in the storage area was important with respect to sampling. The samples were later weighed and stored individually in 0.0029-cm thick clear plastic bags measuring 16.5 cm x 15.0 cm. Data were analyzed by two-way ANOVA and Duncan's multiple range test for differences in contamination (hairs, droppings, urine, and kibbled seeds) between rows and columns.

All the wheat was removed from the frame and thoroughly mixed and 10 random samples of approximately 25 g each were drawn for contamination studies and stored as above. After that the wheat was weighed to determine consumption losses. The method of Laakso et al (1956) was used to determine what portion of each wheat seed sample was contaminated by urine. One seed was placed in each of the 702 depressions of a large clear plastic spot plate. Clean wheat seeds, 10 in number, from the original bags were placed in the remaining 10 depressions to serve as the control. Seeds were then treated with the urease solution (10 mg of TYPE 1X at pH 6.8 jack bean urease, Sigma Chemical Co.[1] in 100 ml of phosphate buffered solution) for 5 minutes, followed by the addition of Nessler's reagent. A yellow complex formed with liberation of ammonia if urea was present. The droppings, hairs, kibbled seeds, and the few remaining whole seeds in the plastic bags were examined under a three-diopter lens where each dropping, hair, and kibbled or broken seed was counted.

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RESULTS

The amount of wheat removed from the feeders daily by the original 12 mice during the initial four-week period was fairly constant, averaging 42.8 g per day (SD 7.94) or 3.6 g per mouse per day. During the same period, water consumption averaged 21.4 ml per day (SD 1.70) or 1.8 ml per mouse per day. During the 150 day simulated storage period, water consumption averaged 35.7 ml per day (SD 10.4). Temperatures within the storage facility increased about 8°C during the summer months which probably contributed to increased water consumption in the summer over the baseline period. Evaporation during each period was measured and found to be negligible (less than 1 ml per day).

A total of 5.584 kg of wheat was removed from the simulated storage container during the 150-day period. An estimated 340 g of whole, broken, and kibbled grain mixed with droppings were scattered over the floor area; an average spillage of 2.3 g per day. Subtracting the spillage from the amount of grain removed from the frame shows that about 35 g were eaten per day.

Based on our samples of the upper 2.5 cm of grain, we estimated 434 droppings and 1,204 hairs per kg. Considering the samples from the upper 2.5 cm representative of the "surface area", we estimated an average of 220 droppings and 611 hairs per square meter. Urine was found on 65 percent of the sampled wheat seeds in the upper 2.5 cm.

Two-way ANOVAs ($P = .02$) showed significant differences in row by column interactions for hair in the upper 2.5 cm of the grain. The number of droppings in the upper 2.5 cm was highly significant in both columns and rows, but showed a nonsignificant interaction. Broken and/or kibbled seed analysis show only slight significance in row and column effects. There were no significant differences on the number of urine-contaminated seeds with respect to location. Duncan's multiple range test ($P = .05$) did not show any consistent patterns with respect to locations of droppings, hairs, or broken and kibbled seed. However, Tables 1 and 2 showed identical locations when the mean number of droppings and mean number of hairs in the upper 2.5 cm were used as measures of the six most active and six least active areas sampled.

Based on our samples of the total wheat seeds (109 kg), we estimated 173 droppings and 454 hairs per kg and 55 percent of the wheat seed contaminated with urine. Broken and kibbled seed averaged 38.0 (SD 7.1) to the sample and 10 control samples averaged 27.2 (SD 4.7) broken seed to the sample.

During the 150-day period, 11 animals were found dead, only two of which (1 male and 1 female) were identifiable as members of the original stocked population.

After the 150-day period, 15 live and two more dead mice were found. Three females of the original stock were alive. Three adult males, one adult female, three juvenile males, and five juvenile females, all unmarked, were found. The two carcasses found were unidentifiable in either sex or marking.

DISCUSSION

In this study, the methods developed to quantify the samples for contamination are simple and rapid with an acceptable level of precision. We found it best to divide each 702-seed sample into thirds. We placed one drop of urease solution in about one-third of the 702 depressions, then placed a wheat seed in each, followed by one drop of Nessler's. This method was necessary because we found that if the seed remained in the urease for the length of time required to do all 702 seeds, much of the urease solution was absorbed by the seed (swelling it to fill the depression), and additional solution was lost to evaporation. The use of plastic bags to count droppings, hairs, and broken seeds under a three-diopter lens was excellent. After some experience, one could process each sample in about 45 minutes.

The method used to estimate consumption in this study obviously is unsuitable for field loss assessments. A population estimate based on capture/recapture (e.g., "Lincoln index," or the method of Otis et al, 1978) and/or other measures such as water consumption or percent of urine-contaminated seeds will be required. Additional tests are currently underway to develop consumption method estimates.

Our population growth rate behaved much like that reported by Southwick (1955). His six populations, starting with four pairs of house mice each in a 150 ft^2 pen, did not substantially increase until after seven months. Our consumption data and final population data are in agreement with that given by Southwick (1955) of 0.17 g per mouse gram per day, and by Greaves (1978) of 15 percent of their body weight per day. Using Southwick's consumption value and the average weight of our original 12 mice (14.4 g), we found an average population of 15 mice during the 150-day period, and using Greaves consumption value and average body weight of 14.4 g, we obtained an average population of 16 mice for the 150-day period.

Urine contamination of the wheat seeds was 11 times greater than wheat seed consumption (55%/5%). Presumably, in bulk storage of large quantities of grain at some depth rodent urine could not be a contaminant because the urine would be absorbed by the grain above that depth. Since only a 10 percent difference in urine contamination was found between the total and the upper 2.5-cm samples, deeper samples will probably be required. In the field, however, these determinations will depend on factors such as surface area, depth of grain, length of storage, and density of rodents.

The sampling pattern used here shows more contamination at the corners of the storage container, somewhat less on the sides, and still less in the center, as shown in Tables 1 and 2. Consequently, the sampling pattern used in the field will depend on the size and configuration of the stored grain. The number of samples taken met the objectives of our study, but in the field the number of samples will be determined by the surface area of the stored grain. The development of sampling criteria is an objective to be addressed in our future studies.

Tests are currently underway to evaluate the validity of the above assumptions relating to grain depth, surface area, population density, length of storage, and method of bulk storage.

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[1] Reference to trade names does not imply endorsement by the U.S. Government.

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Table 1
Mean number of droppings by location.

Rows/Col.	1	2	3	4
A	21.0*	14.5*	[8.5]	21.0*
B	10.5*	[3.5]	[1.5]	[9.5]
C	20.0*	[7.0]	[4.0]	15.0*

* = 6 highest number of droppings and [ ] = 6 lowest.

Table 2
Mean number of hairs by location.

Rows/Col.	1	2	3	4
A	42.0*	34.5*	[33.0]	34.0*
B	42.5*	[6.0]	[21.5]	[32.0]
C	37.5*	[13.0]	[29.0]	54.5*

* = 6 highest number of droppings and [ ] = 6 lowest.